93. The composition of claim 78 wherein said detectable proximity-sensor peptide is a synthetic oligopeptide comprising a fluorescent amino acid derivative.

94. The composition of claim 78 as set forth in Figure 5A (SEQ ID NO: 8).

95. The composition as shown in SEQ ID NO: 9. = 50

Please cancel claims 15-32 and 34-77.

See Interview Summary page #24

REMARKS

The July 3, 2002 Official Action has been carefully considered. In view of the amendments presented herewith and the following remarks, favorable reconsideration and allowance of this application are respectfully requested.

At the outset, Applicants acknowledge the Examiner's indication that Claim 50 is free of the prior art and is directed to allowable subject matter. The subject matter of claim 50 is now presented in new claim 95.

In the Official Action dated October 5, 2001, the Examiner rejected claims 1-14 under 35 U.S.C. §112, second paragraph as allegedly indefinite for failing to particularly point out and distinctly claim the subject matter regarded as the invention. Claim 1 is allegedly indefinite for reciting the feature "**or a fragment thereof**". Claim 7 is rejected for recitation of the term "**recombinant**" because it is not clear if the term refers to the peptide which has been recombined into a different structure or has been prepared by recombinant means. Claim 8 is rejected because there is improper antecedent basis for the recitation of "**N-terminal cysteine and C-terminal thioester**". Claim 10 is indefinite for including "**BODIPY fluorescein**" for both the acceptor and donor. Claim 14 is rejected for reciting "**a third sensor**"

which is allegedly vague and indefinite because the location and attachment of the third sensor to the peptide are unclear.

At page 5 of the Official Action, claims 1-4, 6-7, and 9-12 stand rejected under U.S.C. §102(e) as allegedly anticipated by, or in the alternative, under U.S.C. §103 as allegedly obvious over Garman (US 5,011,910) or under U.S.C. §102(b) as allegedly anticipated by the disclosures of each of the following references:

1) Krafft et al. EP No. 0 428 000

2) Marshall and Toth U.S. Patent No.: 5,011,910

3) Meldal and Breddam WO 91/16336

4) Maggiora et al. (1992) J. Med Chem. 35: 3727-3730

5) Geoghegan et al. (1993) Bioconjugate Chem. 4: 537-544

6) Carmel et al. (1973) FEBS Letters 30: 11-14

7) Wang et al. (1990) Tetrahedron Letters 31(45): 6493-6496

8) Ashcom and Jacobson (1989) Analytical Biochemistry 176: 261-264

9) Garcia-Echeverria and Rich (1992) FEBS 297: 100-102

10) Pennington and Thornberry (1994) Peptide Research 7(2): 72-76

11) Matayoshi et al. (1990) Science 247: 954-958

12) Latt et al. (1972) Analytical Chemistry 50(1): 56-62

13) Carmel and Yaron (1978) Eur. J. Biochem. 87: 265-273

14) Yaron et al. (1979) Analytical Biochemistry 95: 228-235

15) Boigegrain et al. (1990) C. R. Acad. Sci. Paris 310, Series III: 465-470

16) Oliveira et al. (1992) Analytical Biochemistry 203:

39-46

17) Miki and Iio (1993) The Journal of Biological Chemistry 268(10): 7101-7106

18) Wang and Liang (1994) Biochemical and Biophysical Research Communications 201(2): 835-840

19) Haugland, (1992) Handbook of Fluorescent Probes and Research Chemicals, 5th Edition: 10-13

20) Tsien et al. WO 92/00388

21) Sparks et al. WO 96/31625

22) Wolfman and Hammes (1977) Biochemistry 16(22): 4806-4811

23) Dobryszycki and Kochman (1988) Biochimica et Biophysica Acta 956: 217-223

According to the Examiner, each of the cited references discloses fluorescence resonance energy transfer peptides or polypeptides having acceptors and donors either at opposite ends or distributed within the sequence. The peptides are used variously as sensors of catalytic activity, conformational changes within the protein, or distance measurements within the peptide or protein.

The foregoing constitutes the entirety of the grounds of rejections set forth in the October 5, 2001 Office Action.

In accordance with the present amendment, claims 15-32 and 34-77 have been canceled and new claims 78-95 added. Support for the composition claimed in claim 78 can be found in original claims 1, and at page 6, lines 18-21, wherein it is recited that when practicing the described methods, the conditions of the method will include the composition (substrate), the molecule (enzyme) which acts on the composition to modulate its activity and other reagents or other factors necessary for the activity to occur. Support for the term "modification" is found at page 3, line 18.

Support for the substrate remaining intact is provided by the aggregate of the examples, wherein none of the described substrates were cleaved in response to exposure to enzyme.

Support for new claim 79 is found in original claim 4.

Support for new claim 80 is found in original claim 39 and at page 23, line 16. Support for claim 81 is found in original claim 5. Support for claim 82 is found in original claim 2 and at page 3, lines 14-20. Support for claim 83 is found in original claim 3 and in the specification, for example, at page 3, lines 18-20. Support for claim 84 is also found in original claim 3. Support for new claim 85 is found at page 5, line 14 through page 6, line 7. Support for claims 86 and 87 is found at page 7, lines 7-12. Support for claim 88 is found in original claim 6 and Figure 1. Support for claim 89 is found in original claim 7 and at page 33, lines 15-19. Support for claim 90 is found in original claim 9. Support for claim 91 is found in original claim 10 and at page 8, lines 8-11. Support for claim 92 is found in original claim 11. Support for claim 93 is found in original claim 12. Support for claim 94 is found in original claim 13. As mentioned previously, support for new claim 95 can be found in original claim 50. Applicants respectfully submit that the newly presented claims are fully supported by the disclosure in the present specification, are consistent with Applicants' prior election and do not introduce new matter into the application.

NEWLY PRESENTED CLAIMS 51-75 FULLY COMPLY WITH THE REQUIREMENTS OF 35 U.S.C. §112, SECOND PARAGRAPH

The relevant inquiry in determining compliance with the definiteness requirement of 35 U.S.C. §112, second paragraph, is whether the claim in question sets out and

circumscribes a particular area with a sufficient degree of precision and particularity, such that the metes and bounds of the claimed invention are reasonably clear. In re Moore, 169 U.S.P.Q. 236 (C.C.P.A. 1971).

The definiteness of claim language may not be analyzed in the abstract, but must be considered in light of the supporting specification, with the language in question being accorded the broadest reasonable interpretation consistent with its ordinary usage in the art. In re Morris, 44 U.S.P.Q. 2d 1023, 1027 (Fed. Cir. 1997). See also Ex parte Cole, 223 U.S.P.Q. 94 (Bd. Apps. 1983) (claims are addressed to the person of average skill in a particular art; compliance with §112 must be adjudged from that perspective, not in a vacuum).

Furthermore, it has long been held that the initial burden of establishing a failure to comply with 35 U.S.C. §112, second paragraph, rests upon the Examiner. In rejecting a claim for alleged indefiniteness, therefore, it is incumbent upon the Examiner to establish that one having ordinary skill in the art would not have been able to ascertain the scope of protection defined by the claim when read in light of the supporting specification. Ex parte Cordova, 10 U.S.P.Q. 2d 1949, 1952 (PTO B.P.A.I. 1988).

When the appropriate procedural approach is followed in assessing the claim terminology at issue herein, it is beyond question that Applicant has satisfied the definiteness requirement of §112, second paragraph, with respect to the subject matter of the amended claims.

Original claim 1 has been canceled, and new claim 78 presented in accordance with the present amendment. The Examiner asserts that recitation of the term "or a fragment thereof" in original claim 1 is vague and indefinite. Claim 78 now recites composition comprising "a substrate or functional fragment thereof". One of skill in the art would

be readily apprised of the metes and bounds of the claim, having Applicants' disclosure and claims before them. Applicants respectfully submit that the requirement for functionality removes any ambiguity from the claim. Accordingly, the fragment may be a truncated substrate but must retain biological function, e.g., the ability to function as a substrate for the enzyme. Support for the use of enzymatically active fragments of the target (substrate) is provided in the specification at page 20, lines 16-22 and page 21, lines 20-21.

To overcome the rejection of original claim 7 under 35 U.S.C. §112, new claim 89 is presented which recites that the enzyme is produced recombinantly thereby removing any perceived indefiniteness from the claim.

The phrases "N-terminal cysteine and C-terminal thioester", allegedly lack antecedent basis in claim 8. Claims 8 and 65 have been canceled thereby rendering this ground of rejection moot.

Claim 10 has been rejected under 35 U.S.C. §112, second paragraph, for describing "BODIPY fluorescein" as both the acceptor and donor. Claim 10 has been canceled and new claim 91 submitted in keeping with the Examiner's helpful suggestion to recite "BODIPY FL fluorescein". Support for this amendment is found in the specification at page 4, lines 9-12.

The recitation of "a third sensor" in claim 14 is allegedly vague and indefinite because the location and means of attachment of the third sensor to the peptide are unclear. The claims no longer recite the presence of a third sensor thereby rendering moot this ground of rejection.

In light of the foregoing arguments and amendments, Applicant's respectfully submit that metes and bounds of the present claims are clear and unambiguous. Accordingly, Applicant's request that the rejections under 35 U.S.C. §112

second paragraph be withdrawn.

NEWLY PRESENTED CLAIMS 78-95 ARE NOT RENDERED OBVIOUS BY GARMAN

All claim recitations must be considered in determining non-obviousness under 35 U.S.C. §103. In re Sather, 181 U.S.P.Q. 36 (CCPA 1974). It has long been held that when the Examiner disregards specific claim recitations that distinguish over the prior art, the rejection is improper and will be over-turned. In re Glass, 176 U.S.P.Q. 489 (CCPA 1973).

US patent 6,291,201 to Garman et al. describes methods for the preparation of a FRET substrate having donor and acceptor species on opposite sides of a proteolytic cleavage site and wherein the donor and/or acceptor sites are attached to the side chains of the amino acids. Garman teaches that such FRET substrates may be used in assays to identify modulators of protease activity. The FRET substrates described in the '201 patent are cleaved by a protease during the course of the enzymatic reaction. The newly presented claims are directed to the use of FRET labeling and analysis to detect conformational changes in a polypeptide substrate, wherein the substrate is modified but not proteolytically cleaved as a consequence of the activity of the enzyme.

Garman et al. is silent with regard to the use of FRET pairs to assess conformational alterations that occur as a result of a substrate modification that does not include cleavage of the substrate. It is particularly noteworthy that Garman et al. do not teach or suggest the use of the described FRET pairs to assess phosphorylation and dephosphorylation of kinase substrates as claimed in new claims 79-84. In light of the foregoing, Applicants submit that the Examiner has failed to establish a prima facie case of obviousness based on the subject matter of the newly presented claims. Accordingly,

Applicants request that the rejection under 35 U.S.C. §103 based on Garman be withdrawn.

NEWLY PRESENTED CLAIMS 79-95 ARE NOT ANTICIPATED BY REFERENCES 1-24 UNDER 35 U.S.C. §102

The law is well settled that a rejection under 35 U.S.C. §102 is proper only when the claimed subject matter is identically disclosed or described in the prior art. In re Arkley, 172 U.S.P.Q. 524 (CCPA 1972). Inasmuch as the numerous references cited in support of the §102 rejections set forth in the October 5 Official Action fail to identically describe the subject matter of Applicant's newly presented claims, these rejections cannot stand.

Because the prior art of record fails to describe or suggest the essential aspects of Applicant's compositions for detecting the effect of an enzyme on a substrate, it cannot reasonably be maintained that these references anticipate the presently claimed invention. Specifically, the prior art fails to disclose compositions comprising enzyme substrate which is modified by the enzyme, yet remains intact.

A number of the cited references, including: Garman (6,291,201); Wang et al. (EP 0 428 000 A1); 5,011,910; Meldal and Breddam (WO 91/16336); Maggiora et al.; Geoghegan et al.; Carmel et al.; Wang et al.; Ashcom and Jacobson; Garcia-Echeverria and Rich; Pennington and Thornberry; Matayoshi et al., Latt et al.; Carmel and Yaron; and Wang and Liang, relate to the use of FRET analysis to detect cleavage of a labeled protease substrate, wherein such cleavage provides the means to measure protease activity. New claim 78 calls for a composition comprising a peptide substrate or functional derivative thereof which is converted by enzyme activity from an unmodified to a modified state while leaving the substrate otherwise intact. By contrast, in the composition described in each of the allegedly anticipatory

references, cleavage of a FRET-labeled protease substrate results in a change in the FRET emission spectra which registers that the FRET-labels are no longer present on a contiguous or intact substrate, but rather are present on separate, subfragments of the digested substrate. Protease activity, therefore, results in the generation proteolytic subfragments as will be apparent from the following discussion.

As noted above, in connection with the §103 rejection of claims 1-4, 6, 7, and 9-12, US Patent 6,291,201 to Garman et al. describes methods for the preparation of a FRET substrate having donor and acceptor species on opposite sides of a proteolytic cleavage site wherein the donor and/or acceptor sites are attached via the side chains of amino acids therein. The method further comprises contacting a reactive donor or acceptor species with a polypeptide substrate having the side chains of amino acids therein adapted for reaction with the reactive species and then contacting the substrate so obtained with a corresponding reactive donor or acceptor species. Garman teaches that such methods may be used to prepare novel FRET substrates which may be used in assays to identify modulators of protease activity. This reference is, therefore, directed to the use of FRET labeling and analysis to detect conformational changes in an enzymatic polypeptide or a substrate of the enzymatic polypeptide, wherein the substrate is proteolytically cleaved as a consequence of the activity of the enzymatic polypeptide. Thus, this reference does not anticipate the composition as presently claimed which calls for detecting the effect on an enzyme on a substrate, wherein the enzyme activity produces a modification of the substrate, as opposed to a cleavage of peptide bonds between two substrate amino acids.

Wang et al. (EP 0 428 000 A1) is similar to Garman in that it discusses the use of fluorogenic substrates for the

detection of proteolytic enzyme activity. Thus, this reference does not anticipate the composition as presently claimed because it is directed to the use of FRET labeling and analysis to detect conformational changes in an enzymatic polypeptide or a substrate of the enzymatic polypeptide, wherein the enzyme cleaves the substrate.

US Patent 5,011,910 to Marshall et al. is directed to novel fluorogenic substrates for a retroviral protease having a chemical structure X--Thr-Ile--Nle--Phe(Y)--Gln--Arg--NH_2 wherein X is a fluorogenic group and Y is an acceptor for the fluorogenic group, and their use in a fluorometric method for the detection of retroviral protease activity. Thus, this reference does not identically disclose each and every element of the present claims which call for substrates that remain intact in the presence of active enzyme.

Meldal and Breddam (WO 91/16336) describe fluorogenic peptides and their use in the determination of protease cleaving activities. Specifically, the invention relates to peptides which exhibit intramolecular or internal quenching of the fluorescence brought about by a fluorescent chromaphore (donor group) by another chromophore (acceptor group). Table 1 of this application provides data relating to the cleavage of the disclosed peptide substrates. Thus, this reference does not identically disclose the composition of the invention as it teaches cleavage of fluorogenic substrates.

Maggiora et al. describe a general method for the preparation of internally quenched fluorogenic protease substrates using solid-phase peptide synthesis. The kinetic parameters for hydrolysis of synthetic fluorogenic substrates by renin and HIV proteinase are provided therein. The presently claimed compositions are therefore patentably distinct from those described in Maggiora et al. as they are directed to intact enzyme substrates.

Geoghegan et al. describe double fluorescent tagging of human renin and collagenase substrate peptides. Such tagged substrate peptides are disclosed as having utility for measuring catalytic cleavage of the peptide substrate by the enzymes disclosed. Thus, this reference also fails to identically disclose the compositions of the present invention.

Carmel at el. provide methods with which to quantitate the activity of hydrolytic enzymes which facilitates analysis of the kinetics of the reaction. The method is based on the interruption of non-radiative energy transfer between two chromophores attached to a substrate molecule. Upon excitation of the donor, enzymatic cleavage is followed by monitoring either an increase in the fluorescence of the donor or a decrease in the fluorescence of the acceptor The compositions of the present claims are, therefore, distinguishable over this reference because the recited substrates are modified, but not cleaved as a consequence of enzymatic activity.

Wang et al. describe the design and synthesis of fluorogenic substrates for HIV protease which are based on resonance energy transfer. These substrates permit sensitive and continuous measurement of HIV protease activity, are cleaved by the protease, and therefore do not anticipate the presently claimed composition.

Ashcom and Jacobson describe the preparation of self-quenched fluorogenic substrates for proteolytic enzymes. Cleavage of such fluorogenic substrates results in an increase in fluorescent emission, which provides means to assay the activity of a proteolytic enzyme. Thus, this reference also fails to anticipate the presently claimed composition.

Garcia-Echeverria and Rich provide substrates for measuring the catalytic activity of cysteine proteinases. The rate of papain-mediated hydrolysis was monitored by a

fluorescence continuous assay based on internal resonance energy transfer in the labeled synthetic peptide substrates. Such substrates were used to evaluate the effect of amino acid substitutions on the kinetic parameters of papain catalyzed hydrolysis. Inasmuch as the methods of this reference calls for cleavage of the fluorogenic substrates, it fails to identically disclose the composition of the present invention.

Pennington and Thornberry describe a peptide substrate for interleukin-1β converting enzyme (ICE) comprised of the protease cleavage site situated between two fluorophores that are located at the termini of the molecule. Upon cleavage of the fluorogenic substrate, an increase in fluorescence is observed, which facilitates a continuous assay of ICE activity. This reference, like the other protease directed references cited by the Examiner, fails to identically disclose the composition as presently claimed.

Matayoshi et al. describe novel fluorogenic substrates for assaying retroviral proteases by resonance energy transfer. Inasmuch as cleavage of such fluorogenic substrates provides the means to detect and monitor HIV-1 protease activity, Applicant's claimed compositions are patentably distinct.

Latt et al. describe the synthesis and utility of fluorescent substrates for bovine carboxypeptidase A, an enzyme which cleaves peptide bonds adjacent to carboxy terminal aromatic amino acids. For all the reasons described hereinabove, this reference does not anticipate Applicant's claimed compostions.

Carmel and Yaron describe an intramolecularly quenched fluorescent tripeptide substrate of angiotensin-I-converting enzyme and bacterial dipeptidyl carboxypeptidase. Enzymatic cleavage of the fluorogenic substrate results in a detectable increase in fluorescence emissions. This reference is directed to detection of substrate cleavage as an indicator

of enzyme activity and, therefore, fails to anticipate the present invention for the same reasons discussed hereinabove.

Yaron et al. provide a review of the literature directed to the design and application of fluorogenic substrates for proteolytic enzymes. The fluorogenic substrates are cleaved by protease enzymes as indicated by an increase in fluorescence. Thus, this reference does not anticipate the compositions presently claimed.

Boigegrain et al. describe fluorogenic peptide substrates for the aspartyl protease pepsin. The proteolytic cleavage of the peptide side chain abolishes the internal energy transfer and is detected by an increase in fluorescence emission, which serves as an indicator of enzymatic activity. As expected, a single amino acid change in a peptide substrate rendered the mutated peptide completely inert to pepsin cleavage. However the reference discloses that this mutated peptide was **not** a substrate for the protease under investigation. Thus, this reference also does not anticipate the compositions of the present invention.

Oliveira et al. describe intramolecularly quenched fluorogenic peptide substrates for human renin. Cleavage of the fluorogenic peptide substrates by renin is detected by an increase in the fluorescence emission of the resultant substrate subfragments. The ability of other hydrolytic enzymes (i.e., pig renin and acidic protease) to cleave the fluorogenic renin substrates was also assessed. Thus, this reference, which also requires cleavage of substrate, does not anticipate the composition presently claimed.

Wang and Liang describe fluorogenic substrates which are comprised entirely of α-amino acids. These substrates were used in continuous assays to measure the enzymatic activity of the protease renin. This reference is silent with regard to the use of enzymes which leave their substrates intact and thus, fails to constitute anticipatory prior art.

WO 92/00388 of Tsien et al. relates to labeled proteins which are described as suitable for determining the presence of cAMP, other second messengers, and organic molecules. A composition of matter is provided wherein **two** labeled proteins are associated in one state and substantially disassociated in another, the equilibrium between which is controlled by the free concentration of an analyte. Inasmuch as the disclosed FRET pair members are placed on two separate proteins in an interacting protein pair rather than on a single substrate as presently claimed, the reference does not anticipate the composition of the invention.

Miki and Iio describe the use of fluorescence resonance energy transfer to examine the kinetics of structural changes of reconstituted skeletal muscle thin filaments. As neither of these molecules comprises an active enzyme, this reference clearly fails to anticipate the compositions as presently claimed.

WO 96/31625 to Sparks et al. describes polypeptides having functional domains, the nucleic acid sequences encoding them and methods for isolating either the polypeptides or the nucleic acids. This reference does not describe detectable proximity-sensors for incorporation into the polypeptide containing the functional domain or the recognition unit so as to provide means to detect changes in conformation of either polypeptide, which reflect enzymatic activity. Thus, this reference does not identically disclose the composition as presently claimed.

Wolfman and Hammes describe the use of FRET to determine the impact of different effectors on the distance between two sites in rabbit muscle phosphofructokinase. Thus, this reference discloses modification of the enzyme rather than the substrate. It is noteworthy that the chemical modifications whereby the rabbit muscle phosphofructokinase was fluorescently labelled rendered the enzyme inactive. See

page 4809, first column, first paragraph. In view of the above, this reference does not anticipate the composition as presently claimed which calls for modification of a peptide substrate to assess the activity of an enzyme.

Dobryszycki and Kochman describe the use of FRET labeling of amino acid residues of rabbit muscle aldolase to determine changes in the intramolecular distance between the residues that occur in the enzyme upon a change in temperature. This reference fails to disclose a substrate for aldolase, and consequently does not anticipate the compositions presently claimed.

The Examiner cites Haugland et al. for disclosure of a variety of FRET pairs. Applicants respectfully submit however, that Haugland et al. fail to make up for the above-noted deficiencies of the various references cited under 35 U.S.C. §102.

Given the above-noted patentable distinctions between Applicant's invention and the disclosures of the cited prior art references, the §102 rejections set forth in the October 5, 2001 Official Action cannot be maintained.

In view of the amendments presented herewith and the foregoing remarks, it is respectfully urged that the objections and rejections set forth in the October 5,, 2001 Official Action be withdrawn and that this application be

passed to issue, and such action is earnestly solicited.

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